Comparative evaluation of platelet-rich fibrin, mineral trioxide aggregate, and calcium hydroxide as pulpotomy agents in permanent molars with irreversible pulpitis: A randomized controlled trial

Varun Kumar, Ruchi Juneja, Jigyasa Duhan, Pankaj Sangwan, Sanjay Tewari

Abstract

Background: Pulpotomy has been proposed as an alternative for the management of irreversible pulpitis in permanent molars with closed apices. **Aim:** To compare the performances of calcium hydroxide (CH), mineral trioxide aggregate (MTA), and platelet-rich fibrin (PRF) as pulpotomy agents in mature permanent molars with irreversible pulpitis. **Materials and Methods:** Fifty-four permanent mandibular molars with carious exposure and symptoms of irreversible pulpitis were randomly allocated to three groups, and full pulpotomy was performed using CH, MTA, or PRF as pulpotomy agents. Pain intensity was recorded using numeric rating scale score at baseline, 24 h, 7 days, 6 months, and 1 year. Clinical and radiographic assessments were done at 6 months and 1 year. **Statistical Analysis:** Kruskal–Wallis test and Friedman test were used for intergroup and intragroup comparison of pain scores, respectively. The radiographic outcomes between the three study arms were compared using Chi-square test. **Results:** Clinical success rate was 94.4% at 7 days, which dropped to 85.4% at 12 months. All three agents were equally effective in providing pain relief at all the intervals tested, with no significant difference between them ($P > 0.05$ at all intervals). However, at 6 months and 12 months, 26.2% and 52.4% teeth depicted slight widening of periodontal ligament space. No significant difference was observed between the radiographic success rates observed with the three groups ($P = 0.135$ at 6 months, 0.717 at 12 months). **Conclusion:** Pulpotomy exhibited a high clinical success rate in mature molars with irreversible pulpitis and selection of biomaterial did not affect its outcome.

Keywords: Calcium hydroxide, irreversible pulpitis, mineral trioxide aggregate, platelet-rich fibrin, pulpotomy

Introduction

With better understanding of biological mechanisms and advent of new materials, a new treatment paradigm in endodontics oriented toward preservation and tissue regeneration has evolved.[1] Mature permanent teeth with irreversible pulpitis have traditionally been managed with complete pulpectomy. However, this procedure significantly reduces the survival time of the tooth, with a hazard ratio of 7.4:1.[2] Acknowledging the inherent healing potential of an infection-free pulp, attempts have been made to use pulpotomy as a treatment modality in permanent teeth with complete root development exhibiting symptoms of irreversible pulpitis, where radicular pulp is still healthy. The rationale of preserving the vital pulp is that it can continue to serve the function of protecting the tooth from overload by means of protective feedback mechanism[3] and preventing the fracture by damping property because of the presence of pulp and organic tissue in dentinal tubules.[4] It serves defensive and reparative functions as well.

Views on effectiveness of pulpotomy as a treatment modality in mature permanent teeth are equivocal. It is traditionally recommended to use pulpotomy only in primary teeth or young permanent molars with immature apices.[5] Nevertheless, many recent studies[6-12] have reported clinically acceptable success rates with pulpotomy in mature permanent teeth as well. As early as 1995, Caliskan[6] reported the successful management of 26 vital teeth with periapical involvement using calcium hydroxide (CH) pulpotomy. However, they specifically included teeth with chronic hyperplastic pulpitis in young patients only with

Department of Conservative Dentistry and Endodontics, Post Graduate Institute of Dental Sciences, Rohtak, Haryana, India

Correspondence: Dr. Ruchi Juneja,
259/5, Sarai Mohalla, Rohtak, Haryana, India.
E-mail: ruchijuneja1986@gmail.com

Access this article online	
Quick Response Code:	Website: www.contempclindent.org
	DOI: 10.4103/0976-237X.194107

How to cite this article: Kumar V, Juneja R, Duhan J, Sangwan P, Tewari S. Comparative evaluation of platelet-rich fibrin, mineral trioxide aggregate, and calcium hydroxide as pulpotomy agents in permanent molars with irreversible pulpitis: A randomized controlled trial. Contemp Clin Dent 2016;7:512-8.

the assumption that the inflammatory response is limited to pulp chamber in this condition and radicular pulpal tissue is normal. In a study of 23 permanent molars in children with the age group of 7.6–13.6 years, Qudeimat *et al*.[7] found pulpotomy to be successful clinically and radiographically in all included teeth with symptoms of irreversible pulpitis. Taha *et al*.[8] reported a high success rate of mineral trioxide aggregate (MTA) pulpotomy in carious-exposed permanent molars at 3-year follow-up. Similar results were obtained in a series of clinical trials when pulpotomy was used as treatment modality using MTA or calcium-enriched mixture as pulpotomy agent in mature permanent molars with irreversible pulpitis and success rates were comparable to root canal treatment.[9-12]

The choice of pulpotomy agent is an important decisive factor which can influence the success rate of this technique.[13] CH and MTA have been the most commonly utilized pulpotomy agents for vital pulp therapy.[14] However, owing to disadvantages such as degradation over time, formation of tunnel defects beneath dentinal bridges, and poor sealing, CH is slowly losing its popularity as a first choice agent for pulpotomy.[14] Recent publications reveal an acceptable clinical success rate of MTA pulpotomy in carious-exposed mature permanent molars,[7,8,15,16] but most of them are cohort studies without any control group. Issues have also been raised about the initial cytotoxicity and reduction in the size of pulp chamber due to the formation of reparative dentin deeper to CH or MTA.[17]

Rutherford and Fitzgerald[17] described a future paradigm shift, in which a fibrous connective tissue forms superficial to and not at the expense of the pulp tissue, which subsequently mineralizes to form reparative dentin. Finding a biological pulpotomy agent, which attenuates inflammation and promotes regeneration, has been the focus of researchers for a while. Platelet-rich fibrin (PRF) has been reported to be a promising agent for this purpose.[18] Being strictly autologous, it is expected to be more biocompatible and more kind toward pulp, and hence should elicit minimal or nil inflammatory response when placed directly over the amputated pulp. PRF has a potential to support pulpal healing by moderating pulpal inflammation by the release of healing cytokines such as interleukin (IL)-4 and inhibiting the stimulation of matrix metalloproteinase-1 (MMP-1) and 3 by IL-1b.[18] Hiremath *et al*.[19] first reported the successful management of a mature molar with irreversible pulpitis using PRF as pulpotomy agent. However, no long-term study or clinical trial has evaluated the effectiveness of this protocol.

A systematic review by Aguilar and Linsuwanont[20] in 2011, which included studies on vital pulp therapy in carious-exposed pulps with or without symptoms of irreversible pulpitis, indicated a scarcity of good quality clinical studies comparing effect of MTA and CH on success rate of vital pulp therapy. Studies comparing MTA with other biomaterials are also scarce in number.[9] Hence, this clinical trial was designed to compare the clinical and radiographic success rates of PRF, MTA, and CH as pulpotomy agents in irreversible pulpitis affected permanent mandibular molars with complete root development. The null hypothesis was that there is no significant difference between clinical and radiographic success rate of the three pulpotomy agents.

Materials and Methods

The study was approved by the Ethics Committee of the Institute (ECR/495/Inst/HR/2012). The participants were recruited from the pool of the patients referred to the Department of Conservative Dentistry and Endodontics from December 2011 to November 2012. The follow-up period extended till November 2013. Patients with occlusal caries involving permanent mandibular molar teeth with complete root development and symptoms of irreversible pulpitis, i.e., spontaneous, lingering pain exacerbated by hot and cold fluids, and/or radiating pain were screened. Percussion and pulpal vitality tests, i.e., cold test and electric pulp testing were performed, and radiographs were obtained for these teeth. Only those which responded to cold and electric pulp testing and had no periapical involvement were included in the study. Teeth with periapical widening were excluded from the study. Individuals having systemic diseases, on opioid or steroid therapy, or on any kind of antibiotic medication were excluded from the study. Teeth with marginal periodontitis or crestal bone loss, proximal carious lesions, internal/external resorption, calcified canals, excessive bleeding after access cavity preparation not controlled after several minutes, or no bleeding at all were also excluded from the study.

Before recruitment, the sample size was calculated using Walters method[21] with the assumption of the relatively normal distribution of the sample. With the expectation of a 15% mean difference between the three groups, the minimum number of participants needed to induce statistical significance ($P < 0.05$) was calculated as 38 with a power of 0.80 (13/group). To compensate for the attrition at follow-up, it was decided to enroll sixty individuals.

Informed consent was obtained from all the participants. Simple randomization of the participants to the three study groups was carried out using envelopes containing concealed assignment codes in equal numbers for the three study groups. These envelopes were randomly assigned to the participants by a consultant (not the operator) after obtaining informed consent. Both the study participants and the operator were blinded to the group assigned until deroofing of pulp chamber was complete.

The entire endodontic procedure was performed by a single operator in all the participants. After administering inferior alveolar nerve block with 2% lignocaine containing 1:80,000

adrenaline, the tooth was isolated with rubber dam, caries removed with a sterile round diamond bur in high-speed hand piece, and access cavity was prepared. The coronal pulp was removed up to the level of orifices with sterile round diamond bur and copious irrigation. The pulp wound was carefully irrigated with sterile saline solution to arrest bleeding. Care was taken to minimize the time elapsed between pulp amputation and placement of pulpotomy agent. The teeth were then divided into three groups depending on the pulpotomy agent as follows:

- Group 1: CH group: CH powder (Prevest Denpro, Jammu, India) was mixed with sterile saline on a sterile glass slab and 2 mm thick layer was placed over the exposed pulp tissue. Tooth was temporarily restored with Kalzinol (DPI, Mumbai, Maharashtra, India)
- Group 2: MTA group: ProRoot MTA powder (Dentsply Maillefer, Ballaigues, Switzerland) was mixed with distilled water on a sterile glass slab and approximately 2 mm thick layer was placed over the exposed pulp tissue. A moist cotton pellet was placed over it, and tooth was restored temporarily similar to Group 1
- Group 3: PRF group: PRF preparation was started at the time of starting caries excavation. PRF was prepared according to Choukroun's technique.[22] The pulpal wound was covered with a small piece of PRF and covered with approximately 2 mm thick layer of MTA. A moist cotton pellet was placed over it, and tooth was restored temporarily.

Participants were recalled after 24 h for evaluation of postoperative pain. If symptomatic improvement was reported, temporary restorative material was removed completely, and a layer of resin-modified glass ionomer cement (RMGIC) was placed over CH/MTA. The tooth was finally restored with composite. Teeth with no symptomatic improvement were root canal treated.

The participants were assessed for the intensity of pain after 7 days and were then recalled after a period of 6 months and 1 year for evaluation of clinical and radiographic success. Pain assessment was done by numeric rating scale, for which the participants were asked to rate the pain intensity (PI) in the range between zero and ten. Zero denoted no pain, and ten denoted the most severe PI. Clinical failure was determined by the subjective symptoms as explained by the participants and objective signs as recorded during clinical examination including abscess, swelling, sinus tract, and tenderness associated with the tooth.

The assessment of follow-up radiographs was done by two independent observers who were blind to the group allocation. In case of differences among observers in opinion about the periapical status, discussions were held, and consensus about the decision was obtained. Outcome of radiographic success was classified using a modification of Strindberg's criteria,[23] in which tooth with the normal lamina dura without any sign of periapical periodontitis, absence of root resorption, and calcification was considered a success, and if periodontal widening in radiographs was seen at subsequent follow-up examinations, the case was considered a failure and root canal treatment of the involved tooth was carried out.

Statistical analysis

Demographic data were analyzed using Kruskal–Wallis test. The mean pain intensities at various time intervals between the three treatment groups were compared using Kruskal–Wallis test. For comparison of intragroup pain reduction at different time intervals, Friedman test was used. All the participants who required endodontic treatment before 12-month follow-up appointment as a result of clinical failure were excluded from radiographic analysis. The radiographic outcomes between the three study arms were compared using Chi-square test. Interobserver reliability was measured using kappa.

Results

Twenty participants were allocated in each group. Six individuals (two in CH, one in MTA, and three in PRF group) had to be excluded from the study at the time of pulpotomy because of excessive bleeding during the procedure. Thus, a total of 54 individuals participated in the study. Statistical analysis of demographic data did not reveal any significant difference between the three groups in terms of age ($P = 0.224$) and gender ($P = 0.177$) [Table 1]. Five participants were lost to follow-up at 6-month interval and one at 12-month interval. Three cases which presented with clinical symptoms within 7 days (immediate failure) and four cases which were symptomatic at subsequent follow-up (late failures) were also excluded from statistical analysis. Hence, the data of a total of 41 participants were finally analyzed at 12 months. The distribution of the participants in different groups is presented in Figure 1.

Preoperative PI was statistically similar in the three groups [6.31 ± 1.70 for CH, 6.05 ± 1.47 for MTA, and 6.14 ± 1.46 for PRF, Table 2]. There was a significant reduction in pain score from baseline to all test intervals ($P < 0.005$) in all groups. Statistical analysis revealed no significant difference in the mean PI scores between different study arms at all observation

Table 1: Demographic characteristics of study recruits

Group	Male/female	Mean age (range in years)
CH	8/6	17.82 (14-23)
MTA	11/4	21.20 (14-32)
PRF	5/8	25.81 (14-32)

CH: Calcium hydroxide; MTA: Mineral trioxide aggregate; PRF: Platelet-rich fibrin

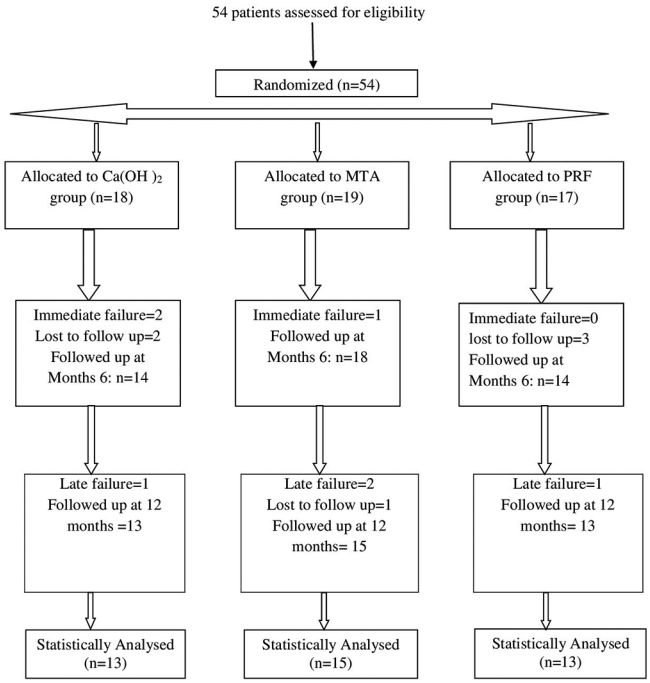

Figure 1: The flow of participants through each stage of trial

periods indicating that all the agents were equally effective in providing pain relief at all the time intervals tested [Table 2].

A high clinical success rate of 93.75% was achieved at 7 days, which dropped to 85.4% at 12 months [Table 3]. No statistically significant difference was observed between radiographic outcomes of the three groups tested [Table 4]. Kappa value was 0.67 which indicated a substantial agreement between the two observers. A remarkable decline in radiographic success rate and hence the overall success rate was observed in all the groups at 6 month follow-up period. This rate dropped further at 12 month follow-up period [Table 5]. None of the teeth, however, had developed well-defined periapical pathology in this interval. Figures 2-4 present radiographs of sample case each from CH, MTA and PRF groups. The overall success rates (combined clinical and radiographic) of CH, MTA, and PRF groups at 1 year were 37.5%, 44.4%, and 35.7%, respectively [Table 5].

Discussion

Increased knowledge of healing and regenerative capability of irreversibly inflamed pulp has prompted researchers to explore less aggressive measures for managing partially inflamed pulp. Wang *et al*.[24] in 2010 reported that the pulp tissue in teeth clinically diagnosed with irreversible pulpitis still has putative cells with stem cell properties. Harnessing the regeneration potential of these cells by giving them a suitable environment may provide a resource for pulp regeneration.

Only mandibular molars were selected for the study so that tooth type does not act as a confounding factor. Teeth having proximal caries were excluded as proximal lesions might have compromised the perfect isolation and good coronal seal which are among the most important factors affecting the success of pulpotomy. Full coronal pulpotomy was selected as it standardized the procedure. A two-layer restoration consisting of RMGIC followed by composite was placed to ensure an effective seal. While RMGIC ensured a good seal with minimal marginal leakage, layer of composite provided compressive and tensile strength and resistance to dissolution.[25]

Table 2: Intergroup comparison of mean pain scores at different intervals

	Mean pain score (values in brackets indicate SD)	*P* (Kruskal-Wallis)
Baseline		
CH	6.31 (1.70)	0.713
MTA	6.05 (1.47)	
PRF	6.14 (1.46)	
24 h		
CH	0.56 (0.89)	0.686
MTA	0.66 (0.84)	
PRF	0.42 (0.64)	
7 days		
CH	1.00 (2.06)	0.179
MTA	0.83 (1.33)	
PRF	0.14 (0.36)	
6 months		
CH	0.64 (1.33)	0.757
MTA	0.52 (1.06)	
PRF	0.50 (1.34)	
1 year		
CH	0.38 (0.65)	0.511
MTA	0.13 (0.35)	
PRF	0.30 (0.63)	

SD: Standard deviation; CH: Calcium hydroxide; MTA: Mineral trioxide aggregate; PRF: Platelet-rich fibrin

Table 3: Clinical success rate of pulpotomy at different intervals

	Success percentage
24 h	100
7 days	93.75
6 months	85.41
1 year	85.4

Table 4: Intergroup comparison of radiographic outcomes (Chi-square test) at 6 months and 12 months intervals

P	
6 months	12 months
0.135	0.717

Table 5: Clinical, radiographic and overall success rates among the three groups

	Clinical success rate at 6 months (%)		Radiographic success rate (%)		Overall success rate (%)	
	6 months	12 months	6 months	12 months	6 months	12 months
CH	81.2	81.2	84.6	46.1	68.75	37.5
MTA	83.3	83.3	80.0	53.3	66.7	44.4
PRF	92.8	92.8	53.8	38.4	50.0	35.7

CH: Calcium hydroxide; MTA: Mineral trioxide aggregate; PRF: Platelet-rich fibrin

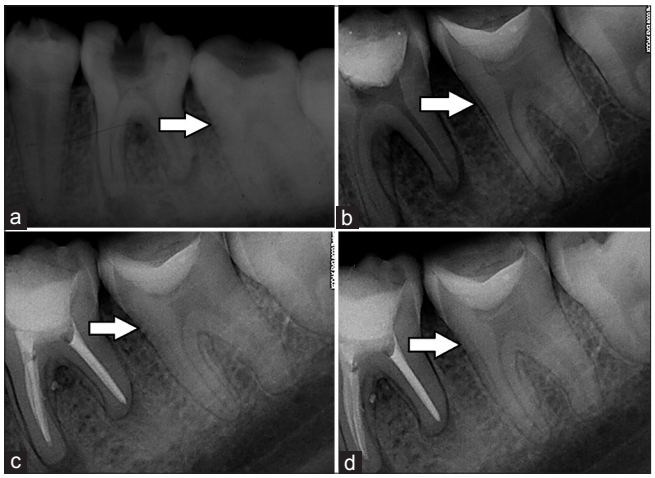

Figure 2: Radiographic evaluation of a case with calcium hydroxide pulpotomy (tooth #37). (a) Preoperative radiograph. (b) Immediate postoperative radiograph after performing pulpotomy. (c) Six months follow-up radiograph. (d) One year follow-up radiograph

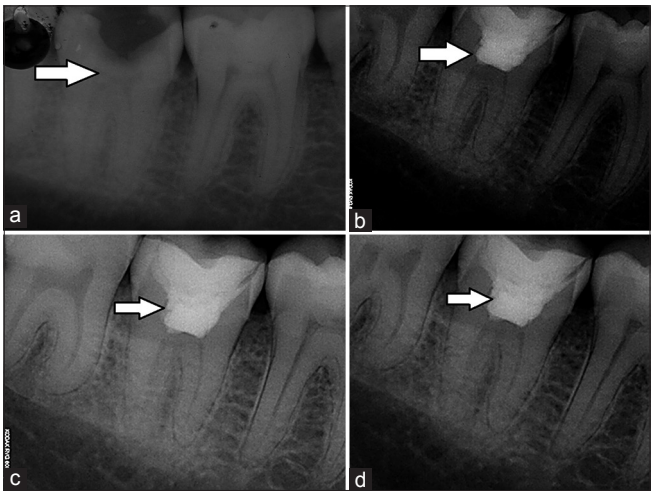

Figure 3: Radiographic evaluation of a case with mineral trioxide aggregate pulpotomy (tooth #47). (a) Preoperative radiograph. (b) Immediate postoperative radiograph after performing pulpotomy. (c) Six months follow-up radiograph. (d) One-year follow-up radiograph

This study reported a good clinical success rate of 85.4% at 12 months. However, when radiographic success was also included, the overall success rate was much lower, being 62.5% and 39.6% at 6 months and 12 months, respectively. As clinical success rate was much higher than radiographic success rate, it can be safely assumed that major cause of failure in pulpotomy treatment is asymptomatic apical periodontitis. The results of the present study corroborate with those of study by McDougal *et al*.[26] who reported clinical success rate of 90% at 6 months and 78% at 12 months. Radiographic success, however, was only 49% of pain-free teeth at 6 months and 42% of pain-free teeth at 12 months. Two common causes generally attributed to a decrease in success rate over time are coronal leakage and presence of residual infection. Although every attempt was made to achieve satisfactory coronal seal in this study, coronal leakage could still be a possible cause of failures, as current restorative materials do not provide a perfect seal. Another limitation was that the crowns were not placed. The low success rate may also be attributed to the use of strict criteria for success.

The status of pulp before vital pulp therapy is also a key factor in determining the success rate of this technique.[5] Although the ability to control bleeding is generally used as an indicator to assess the extent of pulpal inflammation,[27] it may, however, not accurately correlate with the extent of inflammation in all the cases.[28] Thus, many cases having pulpal inflammation at advanced stages may have been included in the study and might have affected the results. Availability of better diagnostic techniques in future may help in the better selection of the cases and hence increased success rate of this technique.

Notably, pulp canal obliteration was not observed in any case at 1-year follow-up. Root canal calcification is one of the reasons why many clinicians choose immediate pulpectomy over attempting pulpotomy as a conservative treatment approach. Mass and Zilberman[29] reported increased pulp calcifications in 28.3% teeth after partial pulpotomy in permanent molars after a mean period of 49 months. However, total pulp obliteration was not found in any of the cases.

PRF was used in the present study considering the advantages of biocompatibility and bioactivity.[30] Huang *et al*.[30] reported that PRF exerted no cytotoxic effect on dental pulp stem cells and each cell maintained its original morphology. PRF also actively participates in pulpal healing by release

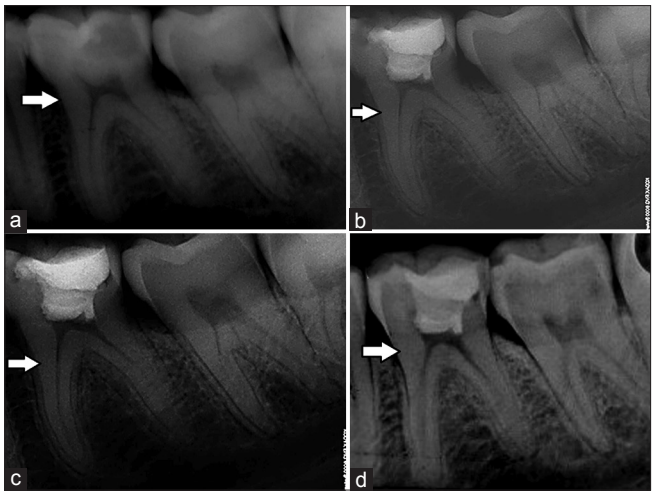

Figure 4: Radiographic evaluation of a case with platelet-rich fibrin pulpotomy (tooth #36). (a) Preoperative radiograph. (b) Immediate postoperative radiograph after performing pulpotomy. (c) Six months follow-up radiograph. (d) One-year follow-up radiograph

of growth factors such as PDGF and Transforming growth factor beta which play an important role in proliferation and differentiation of stem cells.[31] PRF releases healing cytokines such as IL-4 due to activation of a subpopulation of T-cells. This IL-4 supports healing by moderating inflammation. It also inhibits IL-1b-mediated stimulation of MMP-1, MMP-3, and synthesis of prostaglandin E-2.[18] Thus, PRF clot could be of specific advantage in irreversible pulpitis cases with inflamed, and it might be possible to utilize this controlled inflammation as a modulating factor to initiate regeneration in the presence of growth factors and cytokines released by PRF.[32]

The present study reported no statistically significant difference ($P = 0.550$) between clinical and radiographic outcomes in the three groups. Previous studies have reported a superior dentin bridge quality and more predictable results with MTA than with CH.[33,34] The explanation may be provided on the basis that the clinical and radiographic parameters may not provide information about the histological criteria. The quality of bridge formation may not have a direct correlation with clinical success. A histological study may better explain the influence of these three pulpotomy agents on pulpal healing.

It was not possible to include more participants in the current study because of its limited time period, reluctance of patients opting for pulpotomy and strict inclusion criteria adopted. At present, there is insufficient evidence to consider pulpotomy as a permanent treatment modality in cases with irreversible pulpitis. More randomized controlled clinical trials with larger sample size are imperative before a final conclusion can be drawn regarding the usefulness of pulpotomy, the role of bioactive agents and the incidence of pulp canal obliteration in such cases. Another constraint in the present study was the limited diagnostic efficacy of the methods used to establish pulpal diagnosis. Further studies may be carried out using diagnostic methods based on pulpal blood flow for a better case selection.

Conclusion

Pulpotomy as a treatment option for mandibular molars with irreversible pulpitis has an acceptable clinical success rate; however, long-term overall success rate remains questionable. There exists no significant difference between the success rates of CH, MTA, and PRF as pulpotomy agents in teeth with irreversible pulpitis.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Huang GT. Pulp and dentin tissue engineering and regeneration: Current progress. Regen Med 2009;4:697-707.
2. Caplan DJ, Cai J, Yin G, White BA. Root canal filled versus non-root canal filled teeth: A retrospective comparison of survival times. J Public Health Dent 2005;65:90-6.
3. Randow K, Glantz PO. On cantilever loading of vital and non-vital teeth. An experimental clinical study. Acta Odontol Scand 1986;44:271-7.
4. Ou KL, Chang CC, Chang WJ, Lin CT, Chang KJ, Huang HM. Effect of damping properties on fracture resistance of root filled premolar teeth: A dynamic finite element analysis. Int Endod J 2009;42:694-704.
5. Ward J. Vital pulp therapy in cariously exposed permanent teeth and its limitations. Aust Endod J 2002;28:29-37.
6. Caliskan MK. Pulpotomy of carious vital teeth with periapical involvement. Int Endod J 1995;28:172-6.
7. Qudeimat MA, Alyahya A, Hasan AA, Barrieshi-Nusair KM. MTA pulpotomy for permanent molars with clinical signs indicative of irreversible pulpitis: A preliminary study. Int Endod J 2016; [Epub ahead of print].
8. Taha NA, Ahmad MB, Ghanim A. Assessment of mineral trioxide aggregate pulpotomy in mature permanent teeth with carious exposures. Int Endod J 2015; [Epub ahead of print].
9. Asgary S, Eghbal MJ. Treatment outcomes of pulpotomy in permanent molars with irreversible pulpitis using biomaterials: A multicentre randomized controlled trial. Acta Odontol Scand 2013;71:130-6.
10. Asgary S, Eghbal MJ, Ghoddusi J. Two-year results of vital pulp therapy in permanent molars with irreversible pulpitis: An ongoing multicenter randomized clinical trial. Clin Oral Investig 2014;18:635-41.
11. Yazdani S, Jadidfard MP, Tahani B, Kazemian A, Dianat O, Alim Marvasti L. Health technology assessment of CEM pulpotomy in permanent molars with irreversible pulpitis. Iran Endod J 2014;9:23-9.
12. Asgary S, Eghbal MJ, Fazlyab M, Baghban AA, Ghoddusi J. Five-year results of vital pulp therapy in permanent molars with irreversible pulpitis: A non-inferiority multicenter randomized clinical trial. Clin Oral Investig 2015;19:335-41.
13. Swift EJ Jr., Trope M, Ritter A. Vital pulp therapy for the mature

tooth – Can it work? Endod Topics 2003;5:49-56.
14. Bortoluzzi EA, Broon NJ, Bramante CM, Consolaro A, Garcia RB, de Moraes IG, *et al.* Mineral trioxide aggregate with or without calcium chloride in pulpotomy. J Endod 2008;34:172-5.
15. Peng C, Zhao Y, Yang Y, Qin M. Mineral trioxide aggregate pulpotomy for the treatment of immature permanent teeth with irreversible pulpitis: A preliminary clinical study. Zhonghua Kou Qiang Yi Xue Za Zhi 2015;50:715-9.
16. Eghbal MJ, Asgary S, Baglue RA, Parirokh M, Ghoddusi J. MTA pulpotomy of human permanent molars with irreversible pulpitis. Aust Endod J 2009;35:4-8.
17. Rutherford B, Fitzgerald M. A new biological approach to vital pulp therapy. Crit Rev Oral Biol Med 1995;6:218-29.
18. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, *et al.* Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part IV: Clinical effects on tissue healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:e56-60.
19. Hiremath H, Saikalyan S, Kulkarni SS, Hiremath V. Second-generation platelet concentrate (PRF) as a pulpotomy medicament in a permanent molar with pulpitis: A case report. Int Endod J 2012;45:105-12.
20. Aguilar P, Linsuwanont P. Vital pulp therapy in vital permanent teeth with cariously exposed pulp: A systematic review. J Endod 2011;37:581-7.
21. Walters SJ. Sample size and power estimation for studies with health related quality of life outcomes: A comparison of four methods using the SF-36. Health Qual Life Outcomes 2004;2:26.
22. Choukroun J, Adda F, Schoeffler C, Vervelle A. Une opportunite' en paro-implantologie: Le PRF. Implantodontie 2000;42:55-62.
23. Strindberg LL. The dependence of the results of pulp therapy on certain factors. Acta Odontol Scand 1956;14 Suppl 21:1-175.
24. Wang Z, Pan J, Wright JT, Bencharit S, Zhang S, Everett ET, *et al.* Putative stem cells in human dental pulp with irreversible pulpitis: An exploratory study. J Endod 2010;36:820-5.
25. Kleitches AJ, Lemon RR, Jeansonne BG. Coronal microleakage in conservatively restored endodontic access preparations. J Tenn Dent Assoc 1995;75:31-4.
26. McDougal RA, Delano EO, Caplan D, Sigurdsson A, Trope M. Success of an alternative for interim management of irreversible pulpitis. J Am Dent Assoc 2004;135:1707-12.
27. Matsuo T, Nakanishi T, Shimizu H, Ebisu S. A clinical study of direct pulp capping applied to carious-exposed pulps. J Endod 1996;22:551-6.
28. Seltzer S, Bender IB, Ziontz M. The dynamics of pulp inflammation: Correlations between diagnostic data and actual histologic findings in the pulp. Oral Surg Oral Med Oral Pathol 1963;16:969-77.
29. Mass E, Zilberman U. Long-term radiologic pulp evaluation after partial pulpotomy in young permanent molars. Quintessence Int 2011;42:547-54.
30. Huang FM, Yang SF, Zhao JH, Chang YC. Platelet-rich fibrin increases proliferation and differentiation of human dental pulp cells. J Endod 2010;36:1628-32.
31. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, *et al.* Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet-related biologic features. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:e45-50.
32. Cooper PR, Takahashi Y, Graham LW, Simon S, Imazato S, Smith AJ. Inflammation-regeneration interplay in the dentine-pulp complex. J Dent 2010;38:687-97.
33. Asgary S, Eghbal MJ, Parirokh M, Ghanavati F, Rahimi H. A comparative study of histologic response to different pulp capping materials and a novel endodontic cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106:609-14.
34. Nair PN, Duncan HF, Pitt Ford TR, Luder HU. Histological, ultrastructural and quantitative investigations on the response of healthy human pulps to experimental capping with mineral trioxide aggregate: A randomized controlled trial. Int Endod J 2008;41:128-50.